

tetrad array. cDNA constructs carrying wt- β 1a and C-term truncated clones were transiently expressed in mouse β 1-null myotubes and tested for their ability to restore depolarization-induced Ca^{2+} release. Cells were then fixed and freeze-fractured to assess DHPR tetrad arrangement. Our data show that expression of wt- β 1a fully restores EC-coupling, as well as normal DHPR tetrads arrays. Construct β 14, lacking 14 aa of the C-terminal tail of β 1a, still supports normal EC-coupling and also shows normal arrangement of tetrads. On the other hand, β 14 clone containing several Leu/Ala mutations (β 14L/A) that prevent EC-coupling failed to restore tetrad arrays. Immunofluorescence staining confirmed that all clones were expressed and targeted to the plasmalemma. These results suggest that 1) C-term tail of β 1a plays an essential role in permitting and/or maintaining the precise positioning of four DHPRs relative to the four RyR subunits; 2) DHPR positioning in tetrads is essential to EC-coupling and 3) the molecular structure (aa sequence) of the C-term tail is relevant for the role of β 1a in the DHPR-RyR structural and functional relationship.

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Interactions between Dihydropyridine β 1a Subunit and Ryanodine Receptor Isoforms

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Skeletal excitation contraction (EC) coupling requires a physical interaction between the L-type voltage gated dihydropyridine receptor (DHPR) in the transverse tubule membrane and the type 1 ryanodine receptor (RyR1) in the sarcoplasmic reticulum membrane. The C-terminus of the DHPR β 1a subunit influences EC coupling in skeletal myotubes (Beurg et al., *Biophys J.* 1999;77:2953-67, Sheridan et al., *Biophys J.* 2003;84:220-37, Sheridan et al., *Biophys J.* 2004;87:929-42). This may be through a direct interaction with RyR1, as we identified a hydrophobic interaction with L⁴⁹⁶, L⁵⁰⁰ and W⁵⁰³ in the last 35 residues of β 1a that increases RyR1 channel activity in phospholipid bilayers (Rebbeck et al., *Biophys J.* 2011;100:922-30, Karunasekara et al., *FASEB J.* 2012). Additionally, the K³⁴⁹⁵KRRR_R³⁵⁰² motif in a RyR1 fragment (M³²⁰¹-W³⁶⁶¹) pulls down β 1a and facilitates EC coupling (Cheng et al., *PNAS USA.* 2005;102:19225-30). Our preliminary data indicate that substitution of these 6 basic residues with glutamines, abolishes the effect of β 1a on the full length RyR1. We also show that β 1a increases RyR2 activity in a similar manner to RyR1 except for significantly less activation of RyR2 by 10nM β 1a (10 and 100nM β 1a subunit increased RyR2 activity by 1.8- and 2.8-fold, in contrast to 2.6- and 2.8-fold with RyR1). Curiously, this reduced activation of RyR2 by 10nM β 1a is similar to lesser activation of the embryonic alternative spliced (ASI(-)) RyR1 isoform by 10nM β 1a, that lacks residues A³⁴⁸¹-Q³⁴⁸⁵, compared with activation of adult (ASI(+)) RyR1 isoform by 10nM β 1a. Notably, as rabbit RyR2 lacks 4 of the 5 ASI residues. We conclude that β 1a may bind to a hydrophobic pocket conserved in the RyR1 and RyR2 and that this region is influenced by the presence of the alternatively spliced ASI residues and the polybasic K³⁴⁹⁵-R³⁵⁰² motif.

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3D Structural Illumination Microscopy of the Skeletal Muscle Excitation-Contraction Coupling Macromolecular Complex

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To study the structural organization of protein components associated with the membrane compartments involved in skeletal muscle excitation-contraction coupling, we stained enzymatically dissociated mouse FDB fibers with commercially available specific antibodies. Imaging was based on 3 dimensional structured illumination microscopy (3D-SIM, Zeiss Elyra system using a 63x/1.4 Plan-Apochromat lens and an EMCCD camera) or conventional confocal laser scanning microscopy for comparison (Zeiss LSM700). We investigated the localization pattern and distribution of RyR, Cav1.1 and of other protein components involved in excitation-contraction coupling. As expected, imaging of FDB fibers stained with anti-RyR Ab by conventional confocal microscopy revealed highly ordered band-line structures regularly distributed along the sarcomeres which strongly overlapped with Cav1.1. Analysis of the same FDB preparation at higher resolution (Dx-y 110-130 nm and Dz 280-340 nm) by 3D-SIM revealed a more distinct pattern of distribution.

RyRs form 100 nm clusters, which are regularly separated and distributed along the longitudinal axis of the fiber. Anti-Cav 1.1 Ab stained clusters having a sarcomeric distribution co-localizing with RyRs. Cav 1.1 Ab also stained structures adjacent to but not overlapping with RyRs. These results suggest the power of the 3D-SIM approach to gain further insight into the structural organization of sarcotubular membranes in normal and diseased condition.

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Super-Resolution Localization and Distribution of Proteins within the Mammalian Couplon

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We have used 3D dSTORM to characterize the distribution and localization of the ryanodine receptor (RyR), the L-type calcium channel (Cav1.2) and the sodium-calcium exchanger (NCX) within the couplon of the rat cardiomyocyte. The images have a resolution of 20nm in X and Y and 50 nm in Z, and cover areas of up to 1200 μm^2 in XY with depths of up to 700nm in Z. In the case of RyR, hundreds of individual clusters could be identified and characterized. The clusters varied greatly in both size and structure; their internal structure showing little evidence of the checkerboard arrangement that has been thought to be predominant. RyR clusters were identified both in the transverse and axial tubules and in some cases could be seen to be separated by a gap typical of the width of a t-tubule. In general, the clusters of Cav1.2 were smaller and far denser than their RyR counterparts, with their centers appearing to be tightly packed. NCX was much more widely distributed than either RyR or Cav1.2 and formed a dense carpet along the cell surface with little clustering or identifiable detail. While small isolated clusters of NCX were present in the tubular system, there were other regions where the labeling was more widespread and the clusters were poorly defined.

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TRIC-B Channels Exhibit Labile Gating Properties; Evidence from TRIC-A Knockout Mice

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Trimeric intracellular cation channels (TRIC-A and TRIC-B) are located in the sarcoplasmic/endoplasmic reticulum (SR/ER) of most cells. Identifying the distinct biophysical properties of TRIC-A and TRIC-B is difficult because both channels are present in most tissues, yet this is crucial for delineating their individual physiological roles. Skeletal muscle SR vesicles (LSR) from TRIC-A knockout mice were incorporated into artificial membranes under voltage-clamp conditions as previously described [Pitt et al., 2010, *Biophys. J.* 99, 417-426] and single-channel recordings of native TRIC-B were obtained in symmetrical solutions of 210 mM K-PIPES, pH 7.2. The maximum single-channel conductance of TRIC-B was 197 ± 2 pS (n=32; SEM). TRIC-B channels always exhibited sub-conductance gating states and while these were of a variable nature, the predominant sub-conductance levels were found at 156 ± 3 pS (n=17; SEM), 125 ± 2 pS (n=19; SEM), 96 ± 2 pS (n=19; SEM) and 62 ± 2 pS (n=27; SEM). TRIC-B channel gating was voltage-dependent and channels were inhibited at negative holding potentials. For example, the probability of dwelling in the full open channel level was 0.0478 ± 0.0194 at +30 mV but only 0.0010 ± 0.0008 at -30 mV (n=6; SEM; *p<0.05). Application of 300 mM KCl to the cytosolic channel side produced a parallel shift in the current-voltage relationship and a shift in the reversal potential to approximately -20 mV indicating that TRIC-B is not permeable to anions. Our study demonstrates that the single-channel properties of TRIC-B channels are exceptionally labile. This intrinsic variability may be important for enabling flexible physiological regulation of monovalent cation fluxes across the SR membrane.

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Cardiac Ca^{2+} and Free Radical Disturbances in Mice with Arthritis

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Rheumatoid arthritis (RA) is a common inflammatory disease that afflicts ~1% of the population and is more common in women than in men. Cardiovascular disease is the leading cause of premature mortality in patients with RA. Still,